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Recombinant Human Granulocyte Colony-Stimulating Factor (G-CSF) Combined Conditioning Regimen for Allogeneic Bone Marrow Transplantation (BMT) in Standard-Risk Myeloid Leukemia

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We previously suggested that using a combined conditioning regimen including rhG-CSF with allogeneic BMT in refractory AML and CML in blast crisis might reduce the rate of relapse and improve disease-free survival, without any major side effects. In this study, we used the same protocol for 10 AML patients in complete remission (CR) and 6 CML patients in the chronic phase (CP). We compared disease-free survival as well as toxic side effects of the regimen with 6 AML patients in CR and 6 CML patients in CP treated with chemoradiotherapy without G-CSF. The conditioning regimen consisted of TBI and high-dose AraC. RhG-CSF was infused continuously at a dose of 5 μg/kg/day, starting 24 hr before the initial dose of total body irradiation (TBI) until the end of AraC therapy. In all 28 cases, there were no early stage deaths due to regimen-related toxicity (RRT). None of the 10 AML cases treated with the G-CSF combined regime relapsed. In 6 AML cases treated conventionally without G-CSF, one patient died of infection and another relapsed. There were no relapses in either CML group. In the combined G-CSF group, one patient died of interstitial pneumonitis 48 days after BMT, while the rest of the CML cases are still alive. There were no relapses with rhG-CSF and no serious adverse effects in terms of RRT, acute graft vs. host disease (GVHD), or leukocyte recovery. Am. J. Hematol. 57: 303–308, 1998. © 1998 Wiley-Liss, Inc.

Key words: granulocyte colony-stimulating factor (G-CSF); allogeneic bone marrow transplantation (BMT); acute myelocytic leukemia (AML); chronic myelogenous leukemia (CML); cytosine arabinoside

INTRODUCTION

In the last 20 years, BMT has helped to greatly improve the prognosis in leukemia. Yet recurrence remains one of the major causes of failure following BMT. One possible approach to reduce the rate of relapse may be to intensify conditioning with chemoradiotherapy. However, the side effects on normal tissue limit the intensity of conditioning therapy, since large amounts of cytotoxic agents and radiation are currently used to prepare patients for BMT [1].

Many myeloid leukemia cells have a functional G-CSF receptor, so when they are cultured in medium containing G-CSF the ratio of cells in the S phase of the cell cycle increases significantly (see ref. [7]). Therefore, G-CSF will reinforce the cytotoxicity of cell-cycle-specific anticancer agents, such as AraC [2]. Our previous experiments showed that G-CSF increased the cytotoxicity of AraC in a murine myeloid leukemia cell line and in

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TABLE I. Patient Characteristics: All the Cases Received Transplants From HLA Identical Sibling Donors*

Initial	Age	Sex	Diagnosis and stage	Prophylaxis of GVHD	Infused cells [$\times 10^8$]
Conventional regimen group					
A.O	15	F	AML-M5 (1st CR)	MTX + CyA	4.4
T.O	35	M	AML-M3 (1st CR)	MTX + CyA	2.1
Y.S	26	M	AML-M5a (1st CR)	MTX + CyA	2.86
M.S	23	F	AML-M3 (1st CR)	MTX + CyA	2.6
M.N	40	F	AML-M4 (1st CR)	MTX + CyA	2.4
M.K	20	F	AML-M3 (1st CR)	MTX + CyA	NR
T.S	21	M	CML (CP)	MTX + CyA	0.1[a]
M.O	27	M	CML (CP)	MTX + CyA	NR
N.T	31	M	CML (CP)	MTX + CyA	2.9
O.M	31	M	CML (CP)	MTX + CyA	0.29[a]
M.K	37	M	CML (CP)	MTX + CyA	3.2
T.K	42	M	CML (CP)	MTX + CyA	2.2
G-CSF combined regimen group					
K.S	23	F	AML-M3 (1st CR)	MTX + CyA	NR
K.T	28	M	AML-M2 (2nd CR)	MTX + CyA	2.2
T.U	26	M	AML-M4 (1st CR)[§]	MTX + CyA	0.0034[a]
M.S	37	F	AML-M3 (1st CR)	MTX + CyA	3.6
Y.I	25	F	AML-M6 (1st CR)	MTX + CyA	0.24[a]
K.S	28	F	AML-M1 (1st CR)	MTX + CyA	3.36
E.O	40	F	AML-M5a (1st CR)	MTX + CyA	4.19
K.I	21	F	AML-M0 (1st CR)	FK506	3.65
M.K	31	F	AML-M3 (2nd CR)	MTX + CyA	4.25
T.W	40	M	AML-M2 (2nd CR)	MTX + CyA	3.2
Y.Y	33	M	CML (CP)	MTX + CyA	1.8
T.T	37	M	CML (CP)	MTX + CyA	0.26[a]
M.S	29	M	CML (CP)	MTX + CyA	1.43
K.I	35	F	CML (CP)	MTX + CyA	2.34
M.I	52	M	CML (CP)	MTX + CyA	4.52
Y.C	21	F	CML (CP)	MTX + CyA	3.21

*There were no significant biases that might influence the outcome, such as the stage of the disease or age at BMT. The number of infused cells with an [a] indicates the number of CFU-GM, while those without an [a] indicate total mononuclear cells. NR, not recorded.

human myeloid leukemia cells in vitro. We made use of this effect in the conditioning regimen used for human allogeneic BMT in refractory myeloid leukemia, and suggested that the co-administration of G-CSF and AraC effectively suppresses relapse without significant side effects. We used this regimen clinically, for several years, to treat both refractory leukemias and standard risk myeloid leukemias. In this paper, we present the results of a new regimen for treating standard risk myeloid leukemia.

PATIENTS AND METHODS

We studied 16 cases of standard risk AML and 12 cases of CML in the chronic phase treated with allogeneic BMT in our hospital between July 1986 and July 1994. The patients' characteristics are shown in Table I. There were no major differences that might influence the outcome of the study. The routine for administering the conditioning chemoradiotherapy has been described elsewhere [2]. In brief, it consisted of TBI (12 Gy) and AraC (3g/sqm, twice a day, for 4 days) with or without G-CSF. The dose of radiation to the lung and abdomen was decreased to 10 and 11 Gy, respectively, to reduce toxicity.

TABLE II. Recovery Dates Following BMT*

	Neutrophils count ($>0.5 \times 10^9$/l)	Reticulocytes count (>0.01)	Platelets count ($>20 \times 10^9$/l)
Conventional group	26.1 ± 6.9	30.7 ± 6.7	26.6 ± 7.6
G-CSF combined group	24.9 ± 9.5	30.3 ± 9.8	24.7 ± 6.8
P	0.975	0.746	0.390

*Six out of 12 patients treated with the conventional regimen and 9 out of 16 treated with the combined G-CSF regimen were given G-CSF after the BMT because of life-threatening infections. Two cases in the conventional regimen group and 3 cases in the combined G-CSF group had a major ABO mismatch. There were very large standard deviations in each group but no significant differences.

We measured the serum G-CSF concentration in two patients in the G-CSF combined group every 12 hr from just before the initial dose of rhG-CSF until 12 hr after completion of the conditioning. The methods used to measure the concentration of G-CSF have been described previously [3]. The number of transplanted cells and other conditions are shown in Table I. For all patients, the donor was an HLA identical sibling. Except for one patient, who was given FK506, short-term doses of MTX

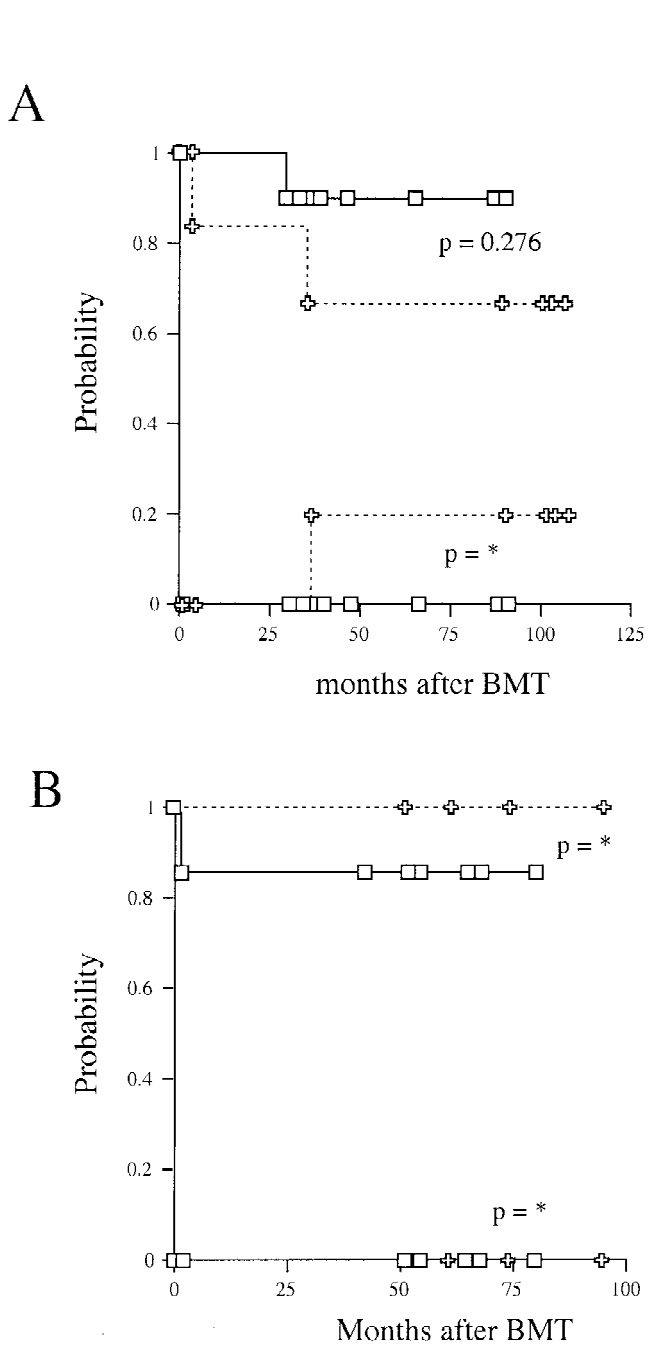

Fig. 1. The *P* value was calculated using the Mantel Cox test. *The calculation could not be completed because no events were observed in one or both groups. A: Disease-free survival and relapse rates of the AML cases, determined using Kaplan and Mayer's method. Open squares indicate the combined G-CSF group while the open crosses with a dotted line indicate the conventional regimen group. The only relapse occurred in the conventional regimen group. B: Disease-free survival and relapse rates for the CML cases. Open squares indicate the combined G-CSF group and the open crosses indicate the conventional regimen group. No patients relapsed in either group.

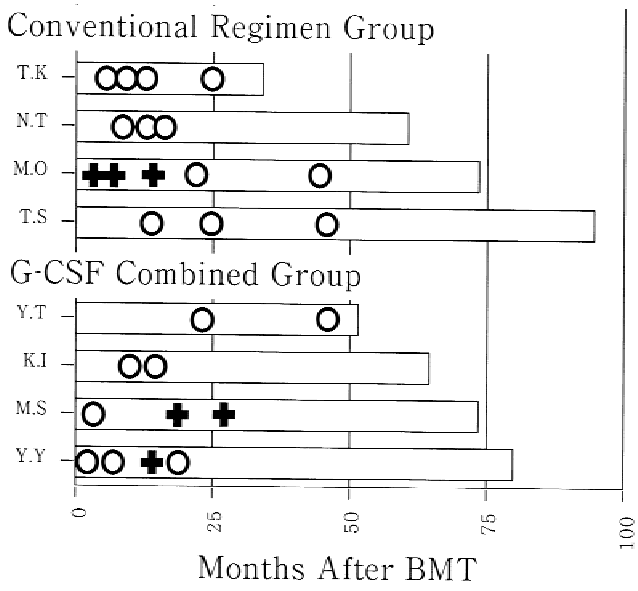

Fig. 2. The minimal number of residual leukemia cells after BMT was determined for the CML cases by the rt-PCR method. Open circles indicate a negative finding (no detectable minimal residual disease) and closed crosses indicate a positive result, in which some leukemia clones were expressed in the marrow.

and CyA were used for prophylaxis of GVHD. We only administered rhG-CSF after the allogeneic BMT to patients who developed a severe bacterial infection while leukocytopenic. We compared disease-free survival, RRT, recovery from myelosuppression, and acute GVHD between the two groups.

RRT was assessed using the World Health Organization grading system [4]. Recovery from leukocytopenia was determined in the following manner. We performed daily peripheral blood cell counts. We defined the granulocyte recovery date as the day on which the first of two consecutive daily measurements made was above 5.0×10^8/L. The erythroid recovery date was defined as the day on which the percentage of reticulocytes was above 1.0%. The platelet recovery date was defined as the last day of platelet transfusion, as transfusion was used to keep the platelet count above 20×10^9/L. The recovery date is given as the mean ± SD for each group. Acute GVHD was diagnosed and graded according to the criteria of Glucksberg et al. [5]. We used the rt-PCR method of bcr/abl fusion messenger RNA to examine the minimal residual disease (MRD) in CML cases, following previously described methods [6].

RESULTS

There were no early deaths from RRT and no graft failure in the 28 cases. All 28 cases were analyzed. As

TABLE III. Acute and Chronic GVHD*

Initial	Acute GVHD Overall	Skin	Gut	Liver	Chronic GVHD
Conventional regimen group					
A.O	0	0	0	0	−
T.O	3	3	3	0	−
Y.S	1	1	0	0	−
M.S	0	0	0	0	−
M.N	0	0	0	0	NR
M.K	0	0	0	0	−
T.S	1	2	0	0	−
M.O	0	0	0	0	+
N.T	1	1	0	0	+
O.M	2	3	1	0	−
M.K	1	2	0	0	−
T.K	1	1	0	0	−
G-CSF combined group					
K.S	1	1	0	0	+
K.T	1	1	0	0	−
T.U	0	0	0	0	−
M.S	3	1	3	0	+
Y.I	1	1	0	0	+
K.S	0	0	0	0	+
E.O	2	3	0	0	+
K.I	0	0	0	0	−
M.K	1	1	0	0	−
T.W	1	1	0	0	+
Y.Y	0	0	0	0	+
T.T	1	0	0	0	−
M.S	0	0	0	0	−
K.I	1	1	0	0	−
M.I	0	0	0	0	+
Y.C	1	1	0	0	−
P	0.904	0.363	0.379	**	0.119

*There were no significant trends in the frequency of acute GVHD. The frequency of chronic GVHD appeared to be higher in the combined G-CSF group, but the difference was not significant ($P = 0.119$).
**As no events were observed in either group, a corrected *P* value could not be calculated.

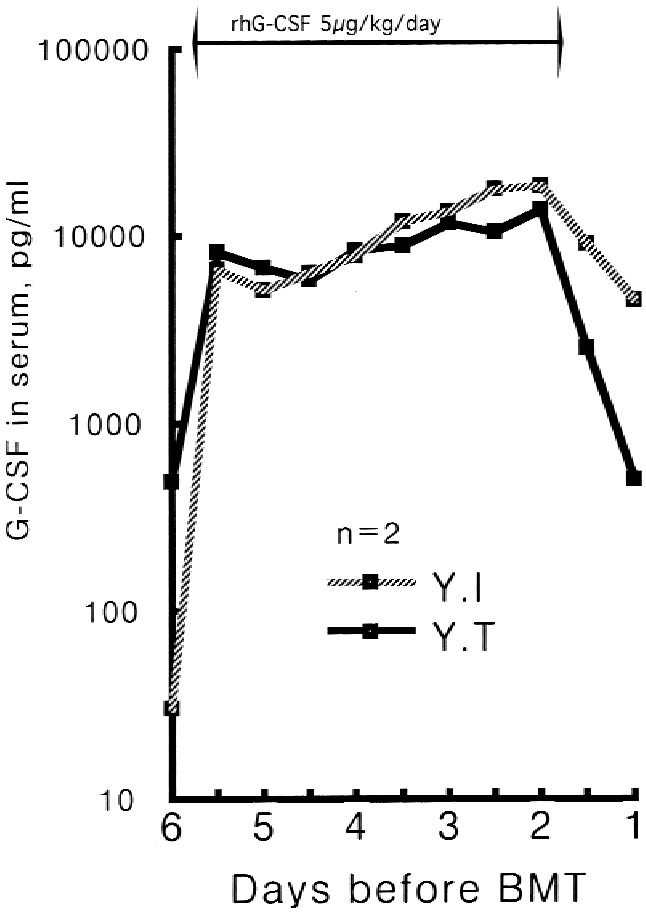

Fig. 3. The serum G-CSF concentration of two patients in the combined G-CSF group was measured every 12 hr. The serum concentrations were maintained at approximately 10 ng/mL. This concentration is sufficient to reinforce the cytotoxicity of jointly administered AraC.

expected, using G-CSF before BMT did not influence the recovery date (Table II).

AML in CR

None of the 10 cases treated on the combined G-CSF regimen relapsed (observation period 29.5–90.3 months). All patients from this group are still alive, except for one, who died from chronic GVHD 29.5 months after BMT (Fig. 1B). Of the six conventionally treated cases (observation period 3.6–107 months), one patient died from an infection, and another relapsed.

CML in CP

There were no relapses in either group. In the combined G-CSF group, one patient died of interstitial pneumonitis 48 days after BMT. The rest are still alive (Fig. 1A).

MRD in CML Cases

A minimum number of residual leukemia cells was studied, using rt-PCR, in 4 of the 6 cases receiving the combined G-CSF regimen (observation period 34.3–95.0 months) and in 4 of the 6 cases on the conventional regimen (observation period 9.5–70.1 months). There were no obvious differences between the two groups (Fig. 2).

Comparison of the Incidence of GVHD

We used Fisher's exact probability test to compare the frequency of chronic GVHD and the Mann-Whitney U-test to compare that of acute GVHD. There were no significant differences between the two groups (Table III).

TABLE IV. Regimen-Related Toxicity in Both Groups*

Case	Stomatitis	Nausea, vomiting	Skin eruption	Hematuria	Bleeding	Diarrhea	Pulmonary	Arrhythmia	Heart failure	Eyes	Consciousness	Peripheral neurolopathy
Conventional regimen group												
A.O	1	2	0	2	1	4	0	1	0	0	0	0
T.O	3	3	2	0	1	4	0	0	0	2	0	0
Y.S	3	4	1	1	1	4	0	0	0	0	0	0
M.S	1	4	0	0	0	4	1	0	0	1	0	0
M.N	1	4	2	0	0	2	0	0	0	4	0	0
M.K	NR	NR	NR	0	NR	NR	NR	NR	0	NR	0	0
T.S	NR	NR	NR	NR	NR	NR	NR	0	0	NR	0	0
M.O	1	4	1	0	1	4	0	0	0	4	0	0
N.T	1	2	1	0	0	4	0	0	0	4	0	0
O.M	1	1	1	1	1	3	0	2	0	2	0	0
M.K	0	3	0	0	0	2	0	0	0	0	0	0
T.K	1	2	2	1	1	2	1	0	0	0	0	1
G-CSF combined group												
K.S	1	3	2	1	1	3	0	0	0	2	0	0
K.T	NR	NR	NR	NR	NR	NR	NR	0	0	NR	0	0
T.U	NR	NR	NR	NR	NR	NR	NR	0	0	NR	0	0
M.S	1	2	1	0	1	4	0	0	0	0	0	0
Y.I	1	4	1	0	1	4	0	0	0	4	0	0
K.S	1	3	1	1	1	4	0	0	0	0	0	0
E.O	1	2	0	0	0	1	0	0	0	3	0	0
K.I	2	2	2	0	0	2	0	0	0	1	0	0
M.K	3	2	3	0	0	3	0	0	0	2	0	1
T.W	2	3	2	1	0	2	0	0	0	3	0	0
Y.Y	0	4	2	0	0	4	0	0	0	3	0	0
T.T	4	3	4	1	0	4	4	3	3	0	2	0
M.S	1	3	1	0	0	4	2	1	2	1	0	0
K.I	4	3	0	0	0	3	0	0	0	1	0	0
M.I	1	3	1	1	1	2	0	0	0	0	1	0
Y.C	2	2	0	0	0	0	0	0	0	3	0	0
P	0.270	0.598	0.561	0.621	0.197	0.325	0.794	0.696	0.239	0.864	0.239	0.768

*No significant differences were observed.

Adverse Effects

In both groups, patients frequently complained of gastro-entero symptoms, such as nausea, vomiting, or diarrhea, and visual disturbances caused by AraC. The differences between groups were not significant using the Mann-Whitney U-test.

Serum Concentration of hG-CSF

As we previously suggested on the basis of in vitro studies, G-CSF promotes the anti-leukemic effect of AraC when the concentration is maintained at about 10 ng/mL, or at least 2 ng/mL. The serum concentrations of hG-CSF were maintained at approximately 10 ng/mL during G-CSF and AraC administration (Fig. 3). Therefore, the amount of G-CSF administered reached the target levels (TBL TU).

DISCUSSION

In the past 20 years, allogeneic BMT has become well established as a therapy for leukemia. When allogeneic BMT is used for the first CR in AML, 50–60% of patients become disease-free and survive for the long term. In refractory AML, only 10–20% of patients experience long-term survival. Relapse is one of the most common problems remaining to be solved in BMT. A more intense conditioning regimen may have higher antileukemic effects, but will also have more adverse effects on nonhematopoietic organs, resulting in a lower survival rate [1]. This trial may offer some hope for resolving this dilemma.

Many human myeloid leukemia cells express functional G-CSF receptors that mediate cell proliferation. If these cells are cultured with human G-CSF, significant numbers enter the S phase in the cell cycle from late G1 phase to S phase [7]. G-CSF also stimulates the proliferation of murine myeloid leukemia cells and NFS60 cells in a dose-dependent fashion, measured by tritium-labeled thymidine uptake [2]. Therefore, G-CSF increases the cytotoxicity of cell-cycle-specific anticancer agents such as AraC. A dose of G-CSF greater than 2 ng/mL significantly increased the cytotoxicity of AraC at different concentrations in both NFS60 cells and most human AML cells. The serum G-CSF concentration during the conditioning regimen was 10 ng/mL, so the dose

and method we used to administer G-CSF in this study and a previous one [2] must be effective. Since G-CSF acts through a receptor expressed on the surface of hematopoietic cells, the placenta, and most myeloid leukemia cells [8], in practice it would only reinforce the activity of AraC in hematopoietic and myeloid leukemia cells.

The rhG-CSF-combined conditioning regimen for allogeneic BMT can potentially help prolong remission after allogeneic BMT and improve the DFS rate in refractory AML [2]. In this study, we used the same regimen as used for AML to treat AML in CR and CML in CP. These results do not show any significant differences in the relapse rate because the sample size is too small and there are very few relapses in either group. However, it is worth noting that there were no relapses in the combined G-CSF group. The follow-up period has been reasonably long (30 to 90 months), and no patients have developed recurrent disease. We can conclude that G-CSF did not stimulate the residual leukemia cells. The combination with G-CSF may increase toxicity to leukemic cells without any of the serious side effects mentioned above. Further testing of this conditioning regime on a larger number of patients is merited.

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